22 RECORDING A MUSCLE TWITCH IN FROG

22.1 INTRODUCTION

In Unit 6 of Block-2 LSE-05 you have learnt about muscles contraction. In the body, skeletal muscles are stimulated to contract by somatic motor nerves through nerve impulses. The nerve cells that stimulate the skeletal muscles are known as somatic motor neurons.These cells as you know, are located in the brain or spinal cord but their axons leave the brain via the cranial nerves and the spinal cord via the spinal nerves known as peripheral motor nerves.

Upon reaching the skeletal muscles the nerve fibres branch and innervate several individual muscle fibres. The connection between the nerve fibre and skeletal muscle is called a neuromuscular junction.

The nerve impulses that reach the neuromuscular junction cause the release of a neurotransmitter acetylcholine from the nerve ending. Acetylcholine combines with the receptors on the muscle fibre membrane causing it to generate and conduct an impulse. The impulse stimulates the release of Ca^{++} and cause the contraction of the muscle fibre.

The skeletal muscles can be stimulated to contract by applying an electric current to the nerve or to the muscle directly. When a single stimulus of sufficient strength is applied, the resultant contraction is know as **twitch**. This can be recorded on an instrument known as **kymograph**. The recording of a skeletal muscle twitch indicates a period between the time the stimulus is applied and the beginning of the contractile response. This interval of time is the latent period. The latent period represents the time required for the electrical current to spread through the tissue and muscle and the release of Ca^{++} in the muscle fibre. Following the latent period is the contractile phase in which the muscle shortens and the relaxation phase, in which the muscle returns to its original length.

In this experiment you will make a preparation of the gastrocnemius muscle-sciatic nerve and record the muscle twitch by stimulating the preparation electrically.

Objectives

After performing this experiment you should be able to:

- make a gastrocnemius muscle — sciatic nerve preparation from the frog.
- record a skeletal muscle twitch and determine the time for latent period, contraction and relaxation phases.

22.2 MATERIALS REQUIRED

Live Frog

Dissection Tray

Dissection Kit

Thread

Bone cutter

Frog Ringer solution

50ml beaker

Cotton

20% urethane, injection syringe and needle

Kymorgraph Recording system having

Kymograph apparatus

1.5 volt dry cell (stimulator) with electrodes attached to it

Muscle lever

Double hook

Femur clamp and stand

5 gram weight

Recording stylus

22.3 PROCEDURE

This exercise would be done in two parts. In the first part you will assemble the kymograph apparatus for recording of the muscle twitch.

In the second part you will carefully dissect and obtain a preparation of the gastrocnemius muscle and sciatic nerve of frog.

Setting up the Kymograph Apparatus

Your counsellor will get ready the recording instrument. This instrument consists of a revolving drum mounted on a suitable motor with adjustable speed (Fig. 22.1). A soot covered recording paper is pasted on the drum. A femur clamp is fitted to a stand to hold the nerve muscle preparation. The electric stimulator used in this experiment is made by soldering two electric wires one on each side of a 1.5 dry cell. The two electric wires are fitted with simple electrodes.

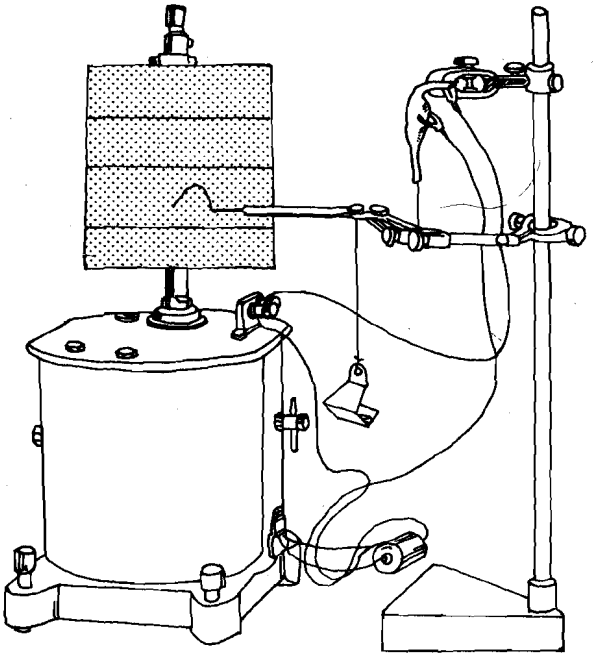

Fig. 22.1 : A kymograph setup for recording a muscle twitch.

A. Nerve-muscle Preparation

(Work in groups of four students)

1. Before you start the dissection, anesthetise the frog by injecting 2.5ml of 20% urethane intramuscularly. This would quieten the frog. Alternately you could immobalise the frog by pithing.

2. For pithing you should hold the frog in your left hand with the head bent forwards. Run the tip of a dissecting needle straight over the frog's head in the midline, until a depression is felt at the rear of the skull. Insert the point of the dissecting needle at this location which will cut the spinal cord from the brain. Aim the needle

forward and destroy the brain by rotating the tip of the needle from side to side. The animal would now be immobile and can be used for dissection.

3. Pin the frog on a dissection tray. Expose the body cavity of the animal and carefully remove the visceral organs. While removing the kidneys take care not to damage the nerves. Now you can see the vertebral column and the spinal nerves attached to it on either side. Always keep the dissection wet using the frog's Ringer solution.
4. Using a bone cutter, cut the pelvic girdle on one side to separate the two femurs.
5. Remove the skin from one of the hind legs by making an incision in the skin around the thigh where it joins the body. Peel the skin down to the toes using a forceps or by hand, peeling it off like a glove. Remove the thigh muscles and connective tissue, and trace the sciatic nerve from the spinal cord to the knee joint. Do not cut the structures of the knee joint or damage the origin of the gastrocnemius muscle.
6. Free the Achilles tendon and gastrocnemius muscle from the surrounding tissues. Tie a strong thread around the Achilles tendon and cut distal to the thread, close to the foot. Using a bone cutter cut the femoral bone near the knee. Also cut the vertebral column above the below the sciatic nerve. Carefully remove the piece of vertebral column to which the nerve is attached.
7. Holding the thread remove the muscle upwards near the tibia. Cut the tibial bone close to the knee joint.

Your final perpetration should consist of a piece of the vertebral column attached to the sciatic nerve, the knee joint with a piece of tibia and femur attached to it and the gastrocnemius muscle with a thread around the tendon. (Fig. 22.2). Throughout the dissection you must take care not to damage the nerve and also to keep the nerve muscle preparation wet by using cotton soaked in frog's Ringer solution.

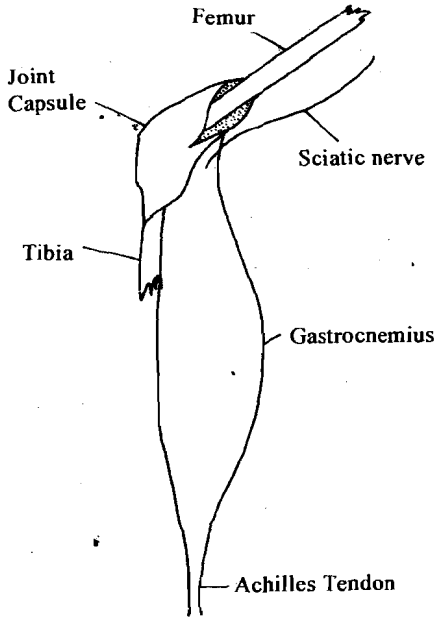

Fig. 22.2 : Gastrocnemius muscle — sciatic nerve preparation.

B. Recording of the muscle-twitch

1. Place the femur in the femur clamp and make sure that the muscle is suspended vertically, directly above the point where it will be attached to the muscle lever.
2. Hook the thread to the lever with a double hook and to the other end of the hook add a 5g weight. This arrangement will keep the lever in horizontal position and the muscle and thread in a vertical position.

3. Clamp the muscle is such a way that the distance between the fulcrum and the tendon attachment would raise the lever by about 2 inches for every contraction of the muscle. Keep the preparation moist by using a cotton soaked in frog's Ringer solution.
4. Attach the writing stylus to the horizontal lever is such a way that it just touches the drum without affecting its speed.Set the motor at a speed of 32 cm/sec. Now your kymograph apparatus is ready for recording the muscle twitch.
5. Position the electrodes near the knee joint. The electrodes should not touch the knee or muscle. Gently lift the sciatic nerve and put it across the electrodes. Deliver a single stimulus from the battery source and record one contraction of the muscle.
6. Drawn on the drum a time tracing for one complete rotation at the same speed. For this purpose, rotate the drum and get a line drawn either at the base or at the top of the drum. Using a stopwatch, at every 10 sec interval mark a point on the line. This can also be done electrically using a tuning-fork with a writing point.

Compare your recording with Fig. 23.3. Identify in the recording the latent period, the contraction phase and relaxation phase.

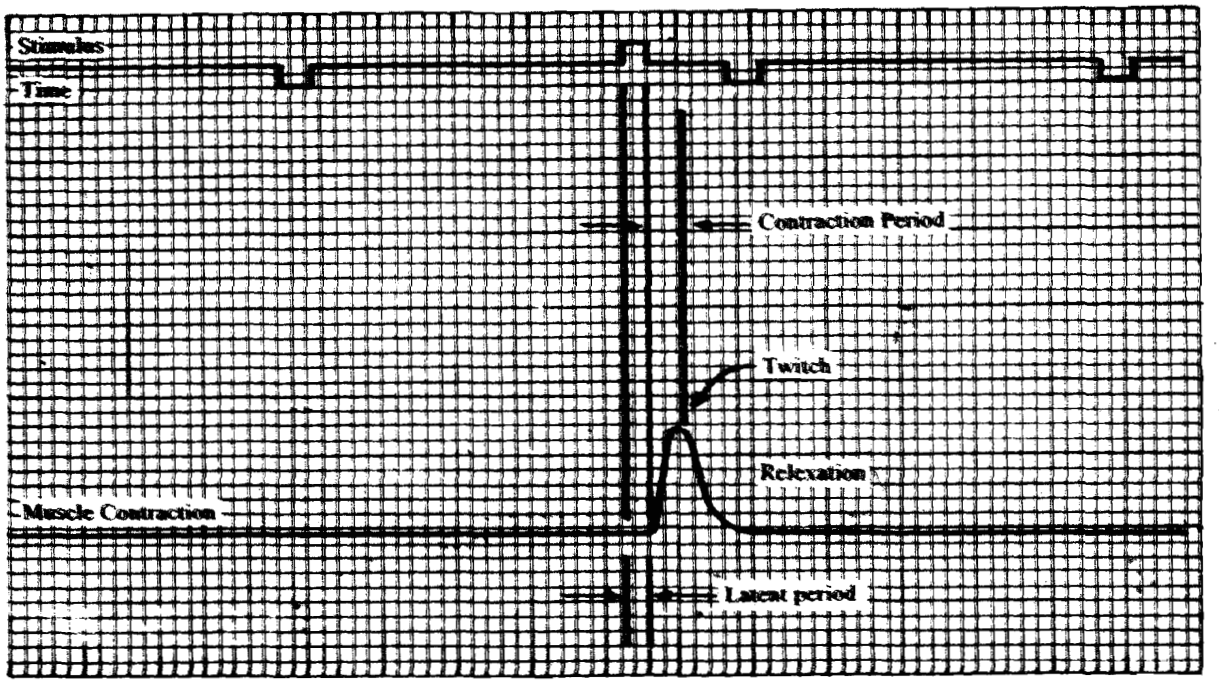

Fig. 22.3 : Recording of a muscle twitch.

SAQ 1. What is meant by a muscle twitch?

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SAQ 2. From the time scale drawn on the drum, can you time the various events such as latent period, the contractile phase and relaxation place?

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SAQ 3. What is the purpose of keeping the nerve muscle preparation wet with frog's Ringer solution?

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SAQ 4. What would happen if the Ringer solution is substituted by distilled water?

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The minimum stimulus required to evoke response in an effector organ is called threshold stimulus. To determine the threshold stimulus, you can perform the following experiment. You would require a setup transformer which can give current ranging from 0 to 5 volts.

Place the electrodes on the nerve muscle preparation and apply a current of varying strengths like 0.5 volt, 1 volt, 1.5 volts and so on. Record the contraction on the kymograph drum at each stimulus applied. Determine the voltage at which the muscle responds. This voltage represents the threshold stimulus. Note the result in your record book.

SAQ 5. On increasing the strength of stimulus above the threshold stimulus is there a significant increase in the strength of the muscle contraction? Give reasons for your answer.

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Activity 1. Pinch the muscle just above the knee using a forceps. Does the muscle respond? If yes, why?

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Activity 2. Touch the nerve with a glass rod dipped in 2% acetic acid. What is the response of the muscle?

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Activity 3. Stimulate the muscle by placing both electrodes on the muscle itself. Does the speed of contraction now differ from the speed of contraction when the nerve was stimulated?

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